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CHOLERA: ITS NATURE, DETECTION, AND PREVENTION.[a]

Prepared by direction of the Surgeon-General.

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Definition.—Asiatic cholera is an acute specific epidemic or endemic disease, due to the presence of the *Vibrio Choleræ Asiaticæ* and of its toxic products (Koch, 1883), presenting usually the symptoms of violent purging, vomiting, muscular cramps, suppression of urine, great fall of blood pressure, subnormal temperature, and collapse.

HISTORY.

It is considered probable by historical students that Asiatic cholera has existed as an endemic disease in the delta of the Ganges for centuries. From this endemic home the disease became epidemic in neighboring districts, and we have positive evidence of epidemics of Asiatic cholera in Goa (1543), Pondicherry (1768), Calcutta (1781), and other parts of India. In the nineteenth century the disease first assumed pandemic proportions and spread from India over Asia, Africa, Europe, and America.

In 1817 the disease spread over all of India and during the period from 1817 to 1837 had become a world disease and a world problem.

Since 1817 six distinct pandemics of cholera are distinguishable.

First pandemic	1817–1823
Second pandemic	1826–1837
Third pandemic	1846–1862
Fourth pandemic	1864–1875
Fifth pandemic	1883–1896
Sixth pandemic	1902–1910

[a] This article was prepared as a further aid in the surveillance over immigrants from cholera-infected countries at their points of destination in the United States. (See Public Health Reports, Oct. 28, 1910, p. 1521.) The prevention of an outbreak of cholera in a community depends primarily upon the prompt detection of the first cases and the methods employed in handling them. A concise description of methods necessary for bacteriologic diagnosis is therefore given, and a detailed account of those preventive measures which should be adopted if the suspected diagnosis is confirmed, or while it is being determined. The paper also makes clear that those associated closely with the bacillus carrier may develop the disease, while the carrier himself may have no symptoms of it.

In addition to bacteriologic studies in the Hygienic Laboratory, Passed Asst. Surg. A. J. McLaughlin had the opportunity of continuing his studies particularly with reference to diagnosis procedures in the Hygienic Institute, Hamburg, and the Institute for Infectious Diseases, Berlin. Subsequently, as acting director of health of the Philippine Islands, he had full charge of cholera suppressive measures during the epidemic of 1908. The experience thus had enables Doctor McLaughlin to present the subject in an authoritative manner.—EDITOR.

The first pandemic (1817 to 1823) spread slowly from the Ganges delta south and east, involving farther India, Java, Borneo, Mauritius, the Philippines, and China. In 1821 it spread north and west by land over the caravan routes to Persia, Mesopotamia, Arabia, Syria, and Astrakhan, and also to Alexandria, Egypt.

The second pandemic (1826 to 1837) had a wider spread. Europe became infected from Persia and Turkey, where infection was carried by returning pilgrims from Mecca. As has always been the case, the disease was carried by more or less sick individuals by land over nearly the whole of Europe, and by sea route to England, Canada, the United States, Cuba, and South America. This pandemic ceased in 1838, and the disease did not appear again until 1846.

The third pandemic of 1846–1862, history repeating itself, spread over the caravan routes from India, Afghanistan, Persia, and Arabia; from Arabia the pilgrims carried it to Turkey and Russia. During this pandemic the disease spread over practically the whole of Europe and North and South America, Central America, and the West Indies. It subsided in 1860.

The fourth great pandemic began in 1863. This pandemic spread more rapidly, due to improved methods of transportation by steamships and railroads. Egypt was infected by pilgrims from Arabia. Instead of the slow spread by pilgrims traveling primitively by caravan, infected individuals were now carried rapidly by steamers from Egypt to Spanish, French, and Italian ports, as well as to Constantinople and Malta. The disease spread rapidly over Italy, France, Spain, Roumania, Turkey, and Russia. In 1866 England and Germany became infected and later Canada, the United States, Central and South America, and the West Indies.

The fifth pandemic began in 1883, took the usual course through Persia and Arabia to Egypt, and from Egypt by sea route to the Mediterranean ports of Italy, France, and Spain. In 1885 it appeared in Japan, and in 1888 spread over the Philippines and Sunda Islands.

In 1892–1893 it spread over practically all of continental Europe. It is estimated that from 1892–1894 800,000 died in Russia of Asiatic cholera, and a terrific outbreak in Hamburg (1892) cost the lives of 8,600 persons. In 1893 the disease reached the port of New York in the person of immigrants from Europe, and a few cases occurred in Jersey City.

The sixth pandemic, which may be said to have persisted up to the present time, began in 1902, and spread through farther India and China to the Philippines. In 1903 it spread as usual by way of Afghanistan, Persia, and Arabia, to Egypt, Syria, Palestine, Asia Minor, and the Black Sea. In 1904 it followed the caravan routes from central Asia to Baku and the Lower Volga. During 1905 it spread over eastern and southern Russia and Poland, and in the same year it appeared in east Prussia, introduced from Russia by raftsmen on the River Vistula. No great spread of the disease has occurred in the German Empire, because of the constant vigilance exercised by the German sanitary authorities and because of the vigorous and thorough prophylaxis employed, although the continued persistence of the disease in Russia has furnished Germany new infection almost yearly. The presence and spread of cholera in Italy, a country from which we receive more immigrants than from any other country, makes the menace of cholera to the United States more direct and threatening than at any time since 1892–3.

The United States quarantine regulations require detention of all immigrants from infected or suspected territory for five days under observation prior to embarkation. These regulations are enforced at foreign ports by American consuls and medical officers of the Public Health and Marine-Hospital Service stationed abroad. Steamship doctors are required to take special precautions in the observation and inspection of immigrants en route to America. At our own ports there is a very rigid and thorough quarantine examination, and later a second examination made under our immigration laws which serves as a double safeguard against the entrance of a person infected with cholera or other dangerous disease.

These precautions would seem to furnish more than adequate protection, yet due to the fact that the infection of cholera may be carried by healthy individuals showing no signs of disease, it is possible for such an individual to enter the United States without detection. Therefore it behooves all health officers to be especially alert and to look with suspicion upon any intestinal disturbance, particularly in individuals recently arrived or associated in any way with newcomers.

The history of these various pandemics is singularly similar. From its endemic home in India by means of the pilgrims and the caravan routes the disease was carried to Afghanistan, Turkistan, Persia, and Arabia. Egypt was usually infected from Arabia. From Egypt, especially after the employment of steamships for sea travel, the infection was rapidly carried to Mediterranean ports of Turkey, Italy, Spain, and France. Pilgrims carried infection from Mecca to Syria, Palestine, Asia Minor, and Russian territory about the Black Sea. Russia also received infection direct from Central Asia over the great caravan routes from Persia, Afghanistan, and Samarcand to the lower Volga and Baku. Infection of Germany and Austria is traceable to Russian and Polish sources. The danger to the United States at present, as in the past, lies in the importation of the infective agent in the person of immigrants from the great European seaports.

ETIOLOGY.

In 1883 Robert Koch demonstrated that Asiatic cholera was an intestinal disease caused by a comma-shaped bacillus found in the contents or walls of the intestine. He demonstrated the connection between an infected cistern and a severe outbreak of cholera. His findings were doubted at first by some, but were soon verified in thousands of cases in later epidemics by other workers. The finding of vibrios in individuals who are not sick does not weaken the etiological significance of the cholera vibrio. It is well known that these bacillus carriers exist in many other diseases, and the development of the disease depends not only upon the presence of the etiological factor but also upon the susceptibility of the individual. Since the perfection of serum diagnosis, especially due to the classical work of Kolle, the degree of illness, the clinical picture, and the morphology and cultural characteristics of the vibrio are of secondary importance in diagnosis, and the application of serum diagnosis determines positively the existence of cholera infection in an individual whether he be very ill, slightly ill, or apparently healthy.

Morphology of the cholera vibrio.—In stained preparations the cholera vibrio is a short slightly curved rod about 1.5 microns in

length and 0.4 microns in width. By juxtaposition of two or more curved organisms, we find spirals, S-shaped, U-shaped, and other forms. Great variation is possible in different strains, of length, thickness, and degree of curvature; some forms having little curve and approaching the ovoid cocco-bacillus type. Great variation from type is more apt to be found in old cultures which have been kept on artificial media for long periods. In these, long threads or spirals are often found, and the curve may be entirely absent. Cultures not more than twenty-four hours old and freshly isolated from stools will be generally found to conform to the type described above.

The motility of the cholera vibrio is remarkable. In a hanging drop they shoot through the field with great rapidity. That the motility is due to a single flagellum has been demonstrated by Kolle and his coworkers; noncholera vibrios have frequently 2 to 6 flagella.

The cholera vibrio does not form spores, hence is easily killed. Forms which did not stain well and light staining spots have been shown by Kitasato, van Ermengem, and others to be sterile involution forms.

Single individuals of different cholera strains may differ greatly. Some individual vibrios are long and slim and very slightly curved, others are short and sharply curved, while some are short and have so little curve that they are almost of the cocco-bacillus type. These variations simply demonstrate that morphology can not be relied upon for diagnosis. All that one can say is that a vibrio is present; its exact identification will depend entirely upon biologic reactions.

Cultural characteristics of the cholera vibrio.—Cholera vibrios are easily cultivated upon the ordinary media, but for practical purposes only peptone and agar media need be considered. Gelatin has been discarded by practically all workers. It has no advantage over agar and its disadvantages are manifest. Upon peptone within from four to eighteen hours the cholera growth is evidenced by turbidity of the media and by a dense cloudy zone near the surface of the liquid. The intensity of this cloudiness depends upon the age of the culture. A distinct surface film forms, but it is delicate and not thick in pure culture, and scarcely perceptible before eighteen hours. Upon agar the cholera colonies are characteristic and easily differentiated with a little practice from *B. coli* and other common intestinal bacteria. Its cultural characteristics are shared, however, by many noncholera vibrios, and cultural characteristics, like morphology, fail to differentiate the cholera from many noncholera vibrios. The colonies of *B. coli* on ordinary agar are whitish and opaque, while the cholera colonies are pale semi-transparent disks which show by transmitted light an opalescent or iridescent quality, which is rarely shown except by vibrios.

Dieudonne's elective blood agar media has the advantage of inhibiting many of the common intestinal and air-borne organisms. Cholera and some other vibrios grow luxuriantly upon it and the colonies have a dirty gray appearance, the color of pus with a tinge of blood in it. Dieudonne's media is of very slight importance to the practiced worker in view of the fact that there is no difficulty in securing isolated cholera colonies on ordinary alkaline agar media with the methods now generally employed.

The "cholera red" reaction.—By adding 3 or 4 drops of concentrated chemically pure sulphuric acid to an eighteen hour peptone or bouillon culture of cholera, a color varying from rose pink to the color

of Burgundy wine is produced. This characteristic is valueless for exact diagnostic purposes because it is also exhibited by many other vibrios.

Failure to produce cholera red should not be charged to the vibrio until the peptone solution has been tested with organisms which are known to produce cholera red. It is said that the presence of glucose in the peptone is responsible for the failure of the reaction. The peptone of Chapoteau seems more reliable than that of Witte for peptone solution intended for the cholera red test.

PATHOLOGY.

The appearance of the cadaver in cholera is charactertistic. Cyanosis is marked. The skin is dry and the abdomen retracted; the eyes are sunken, half closed, and lusterless. Rigor mortis sets in early, and muscular movements especially of the fingers may occur for some hours after death. Upon opening the body the tissues are found to be dry and the serous cavities without fluid. The blood exuding from the organs on section is thick and tarry. The right heart and venous system are engorged with blood. The left heart and arterial system are empty. The skin of the fingers and toes is shriveled; the so-called "washerwoman's fingers." The injection of the small intestine gives it a pinkish color which is very striking by comparison with the large bowel or with normal intestines. Upon opening the peritoneum the intestines will be found to be without luster, resembling "ground glass" and covered with a peculiar sticky material which with the diffuse rosy color of the small intestines is pathognomonic of cholera.

There is usually a parenchymatous nephritis of varying intensity according to the stage of the disease. Parenchymatous changes in other organs may be slight or absent. The changes in the intestine depend upon the duration of the disease. The longer the disease has existed before death the greater the changes in the intestinal tissues. If death takes place in a few hours we have only the rosy flush shown by injection of the small intestine and the intestine filled with a clear fluid in which flakes of mucous and epithelial cells are suspended, or the fluid may be slightly blood tinged. Intestinal contents in cholera have been likened to sago water, rice water, and pea soup. These terms are self-explanatory and represent variations due to death occurring in different stages of the disease. When the disease has existed for some time before death the epithelial lining is denuded, the submucosa red and inflamed, especially around the solitary follicles and Peyer's patches. Section of the intestine shows microscopically the presence of vibrios in the mucosa and as deep as the submucosa. The vibrios will also be found beneath the epithelial lining of the gland ducts. More severe lesions, of a necrotic or diphtheritic character are found in cases of long duration (the so-called "cholera-typhoid"), but these changes are not common and are due to complication and mixed infection with other organisms.

SYMPTOMATOLOGY.

As in all other infections, the clinical picture varies, depending on the severity of the case. We have in Asiatic cholera every gradation from the severest fulminant case of cholera sicca, fatal in a few

hours, to the bacillus carrier who has absolutely no symptoms and whose infection is accidentally discovered. With such variety of types it seems futile to attempt description and classification, as with the exception of the classical type picture description of the other forms would not be a great aid to diagnosis. In typical cases, with vomiting, diarrhea of a rice water character, cramps in the abdomen, legs, and arms, subnormal temperature, loss of voice, failure of the pulse and collapse, the diagnosis is not difficult, and this symptom complex forms a picture once seen never forgotten.

In addition to typical cases, however, we have occasionally cases fatal in a few hours without diarrhea (cholera sicca) and very often atypical cases in which many if not all of the classical symptoms are absent.

These atypical cases are the more dangerous because they frequently end in recovery, and, being unrecognized, serve to spread the disease. They may have no symptom except a diarrhea, which may or may not be choleraic in character. In times of cholera danger the only safeguard is to examine the stools of all such diarrheas. If bacteriological examination of the stools is not feasible, the stools should be treated as infectious for the protection of the public.

Symptoms of typical cholera.—The so-called prodromal symptoms of cholera are too vague to be of any value in diagnosis. Writers speak of premonitory diarrhea, but this symptom, if present, would never suggest cholera, unless vibrios were present in the diarrheal discharge.

In the writer's experience cholera cases are either atypical from the beginning or begin suddenly without noticeable prodromes. Typical cholera begins with profuse watery stools. The fæcal character of the first stools is soon lost and the discharge assumes the appearance of thin rice water with flocculi or granules of mucus suspended therein. The first vomited material may contain food, but later the vomitus is thin and watery, resembling rice water. Muscular cramps in the abdomen and limbs cause great suffering and the spasmodic knotty contraction of muscles is characteristic of the disease. There is a very rapid shrinkage of the soft tissues of the body, due to the enormous loss of fluid, and evidenced by falling in of the cheeks, sunken eyes, shriveled fingers and toes, and general emaciation. There is usually complete suppression of urine and bile. Respirations are rapid and shallow. The body surface is cold and covered with a clammy sweat. The surface temperature falls 4° or 5° below normal, but the rectal temperature may show 38° to 40° C. The pulse becomes rapid, feeble, fluttering, and then imperceptible at the wrist. Cyanosis is marked; the face, and especially the fingers and toe nails, assuming a bluish tint. The voice is reduced to a whisper. These symptoms are sometimes followed by complete collapse and death. This may occur at any time before the expiration of twenty-four hours.

In other cases vomiting and purging cease by adequate treatment and sometimes spontaneously. The body heat returns, the pulse becomes perceptible, then strong again, the secretion of urine begins to be reestablished, and the patient is on the road to recovery. Other cases which do not die in collapse react slowly and pass into a condition which many writers have described as "cholera typhoid." In

this state there is some fever, the shrunken tissues fill out, and the urinary secretion returns. The stools assume a pea-soup character and are very offensive. The urinary secretion returns, but the urine is scanty, albuminous, and contains many casts. From this point the biliary secretion may return and the stools approach the normal type, the albumen and casts diminish, and the quantity of urine increase, the patient progressing to convalescence. On the other hand, from this point if the secretion of urine fails to improve, then any of the symptoms and conditions due to uræmia may be expected, including convulsions, coma, and death.

BACTERIOLOGICAL DIAGNOSIS OF CHOLERA.

In combating cholera, our sheet anchor is the exact bacteriologic diagnosis. Diagnosis by means of the agglutination reaction and Pfeiffer's phenomenon permits us to differentiate cholera from toxic gastro-enteritis, ptomaine poisoning, and other diseases resembling cholera. It further enables us to diagnose Asiatic cholera when the classical symptoms are absent or masked or in those cases in which the patient presents no symptoms whatever (bacillus carriers). In other words, this exact diagnosis obviates the necessity of fighting in the dark, and enables us to concentrate our efforts upon finding and rendering innocuous foci of infection.

The picture of so-called "Cholera nostras," which is probably not due to one, but to many different causes, and the picture of fish, meat, cheese, or ice cream poisoning, may be very like cholera with vomiting, diarrhea, subnormal temperature, loss of pulse, suppression of urine, and collapse. The symptoms given are common to the action of various toxic substances upon the human organism. To attempt differentiation by clinical symptoms alone is always uncertain and in some cases quite impossible. By the bacteriologic methods now in use we have a certain means of differentiation which gives us results within a few hours.

The material for the diagnosis of Asiatic cholera is obtained from the stools of the sick or suspected one, or from the intestinal contents of the dead. If a normal stool can not be obtained, as in the case of a healthy "contact" or person living in the house with a cholera patient, a cathartic such as sulphate of magnesia may be administered. Sometimes with a patient not seriously ill, but whose bowels have been moving freely, it is inadvisable to give cathartics, and yet an annoying delay may occur in waiting for a specimen. In such circumstances pass the largest size catheter or a stomach tube high up in the large bowel. Upon withdrawal the "eye" of the tube will be plugged with mucus scraped from the lining of the bowel, and this can be transferred to media by means of a platinum loop. It must be remembered that this method is only reliable when the patient's bowels have been moving freely just preceding the taking of the specimen. In fatal cases the specimen should be taken from the small intestine[a] at autopsy, or, if complete autopsy is not possible, an incision may be made in the abdomen, a loop of small intestine drawn out of the abdominal cavity, and a section 4 or 5

[a] It is best to cut out two sections of small intestine, one from the middle and the other from the lower portion of the ileum, just above the ileocecal valve.

inches in length should be cut out between ligatures, and brought or sent to the laboratory.

First smears should be made from the fecal material obtained. Flakes of mucus should be selected and smeared upon clean glass slides. After drying in the air and fixing by passing the slide rapidly three or four times through a gas flame, stain for a half minute with carbolfuchsin solution, diluted by the addition of nine times its bulk of water. In cases with typical symptoms, the presence in the stained preparation of a great predominance of vibrios over other organisms is very suggestive of cholera, and the practiced observer will often be willing to risk a diagnosis upon this alone. It is a risk, however, and an unnecessary risk, as verification by agglutination is not difficult and should be carried out in all cases.

It must be borne in mind that in normal and diarrheal feces fine spirilla are found, which, although they do not greatly resemble cholera organisms, being longer, narrower, and less curved, may cause confusion. There are also the so-called cholera-like vibrios, which are morphologically and culturally indistinguishable from cholera, their differentiation being possible only by the agglutination and other biologic tests. It is not known if these are common in America, but they are frequently met with in the Tropics, and the writer isolated twenty different strains of these in Manila from intestinal contents, shallow contaminated wells, and other sources.

Inoculation of cholera peptone media.—From three to six tubes of cholera peptone solution (see Appendix) should be inoculated each with a loopful of the fecal material, selecting a flake of mucus if possible from the most liquid part of the stool. Also add 1 c. c. of fecal material to a flask containing 50 c. c. of peptone solution. Place these tubes and flask in the incubator at 37° C, or, if no incubator is available, place in a warm room and try to maintain the temperature between 27° and 37° C. Examine the tubes after three, six, twelve, and twenty-four hours by making stained smears from the surface. If a thick pellicle forms in this time it is well to avoid it, as other organisms will probably predominate therein. By tilting the tube very carefully toward the horizontal, the pellicle moves away from the lower side of the tube, and a loopful may be secured, without touching the pellicle, from the intensely cloudy zone just below the surface of the liquid. If vibrios are scarce or absent in the smears from the peptone tubes they may be abundant in the 50 c. c. flask of peptone. If the three-hour examination is negative the tubes and flask should be replaced in the thermostat, to be examined again after a growth of six to twelve and twenty-four hours. It is to be remembered that the cholera peptone solution is an elective medium and favors the growth of vibrios, especially in the first eight hours of growth. If vibrios are few in the first peptone tubes after three hours it is wise to make a second series from the first, as well as to permit the original peptone tubes to incubate longer.

Agar plates.—The alkaline cholera agar (see appendix) should be used. For convenient use it should be melted and about 15 c. c. placed in each tube and allowed to solidify with the tube in a slanting position. The plates are made by pouring the contents of one tube, melted in a water bath, into each petri dish. The surface of the agar plates must be dry, and after solidification has taken place, this is best affected by placing them for five minutes in a warming oven

at 60° C., or remove the cover and place with agar surface downward in the thermostat at 37° C. for one hour.

Inoculation of the agar plates should be made direct from the fecal material and as a matter of course from the surface of any peptone tube, from which the stained specimen shows vibrios present. Inoculation of the plates may be made with a bent glass rod, a swab, or with the ordinary platinum loop.

The amount of material used should be one loopful, and three plates should be successively streaked with the same loop without renewing the infected material. In this way isolated colonies are usually obtained in the first plate and always in the second or third.

It is sometimes advisable in making plates direct from stools to add one loopful of the fecal material to 1 c. c. of peptone or bouillon and streak the agar plates from the dilution.

It will be noted that no mention is made of gelatin media or of the growth of vibrios thereon.

Gelatin occupies considerable space in text-books upon cholera, but has been abandoned by all practical workers, and now possesses little more than historic interest or value.

Dieudonne's elective blood agar media (see appendix) was tested by the writer with fresh cholera stools in Manila. It greatly inhibits the growth of colon and other intestinal bacteria, also of the common air-borne yeasts and molds. Cholera and some other vibrios grow luxuriantly thereon, and the colonies may attain microscopic size earlier than upon the ordinary media. At first glance it seems an ideal media, but its importance is lessened by the fact that there is no difficulty in isolating cholera in pure culture with the ordinary cholera peptone and agar now in use.

The agglutination test.—The agar plates are placed in the thermostat at 37°, or kept in a warm room as near that temperature as may be possible.

Within eighteen hours the cholera colonies appear easily distinguishable from those of colon-like organisms by the qualities described above. Given the vibrio colony, it is then only necessary to apply the serum-agglutination test to ascertain if the vibrio is a cholera vibrio or a nonspecific vibrio which resembles it. There may be many isolated colonies upon the plate, and there is always the possibility of cholera-vibrio and noncholera vibrios coexisting; therefore it is often necessary to test many colonies. For routine diagnostic work the following procedure will be found to save time and is the one employed in Manila.

A dilution of 1 to 200 of an agglutinating cholera serum (see appendix) having an agglutinating limit or titer of not less than 1 to 1,000 should be used. A drop of this dilution should be placed at each of three equidistant points upon a clean glass slide. These drops upon the slide are numbered 1, 2, and 3. A portion of colonies correspondingly numbered is transferred from the plate to the drops of diluted serum by means of a straight-pointed platinum wire. The diffuse cloudiness effected in the drops of serum remains permanent in the case of noncholera vibrios, but if the vibrio be cholera the familiar phenomenon of agglutination is macroscopically apparent. The diffuse cloudiness gives place within a few minutes to a clear fluid containing numerous floccules in suspension. The droplets soon dry in

the air and may be fixed and stained when the characteristic vibrios may be seen stained in clumps.

The agglutination phenomenon may be observed microscopically by the hanging drop method, inoculating a drop of diluted serum from a cholera colony in the same manner as described above. For diagnostic purposes, the macroscopic agglutination test is sufficient. Quantitative macroscopic agglutination tests may be made in the following manner:

In small test tubes (2 c. c.) one-half c. c. of dilution of serum varying from 1 to 10 to 1 to 4,000 or up to the limit of serum's agglutinating power. To this quantity of serum one-half c. c. of an emulsion of the vibrio to be tested is added, and the results noted after 1 hour in the thermostat at 37° C., and after an additional 2 hours at room temperature. A smooth emulsion is best prepared by adding to cultures 18 hours old on agar slants, 5 to 8 c. c. of sterile salt solution. With young cultures very little shaking is necessary, and it is never necessary to scrape off the culture, a procedure to be avoided. The test-tube racks should be painted black, to make the reading of results more easy. In the pipette work of delivering quantities (one-half c.c.) of virulent culture in each tube, it is advisable to use cotton plugs in the upper end of the pipettes and to employ a rubber nursing-bottle teat to furnish the necessary power of suction and expulsion. Of course, by adding the equal quantity of culture to the serum dilution, the amount of dilution is multiplied by 2. Thus 1 to 50 becomes 1 to 100, and 1 to 500 becomes 1 to 1,000. Some workers add a loopful of culture to 1 c. c. of the serum dilution, rubbing it up slowly on the side of the test tube. It takes more care and time to effect a smooth suspension in this way, but the readings are made without change in the dilution—1 to 50 remains 1 to 50, etc.

Other diagnostic tests.—For the description of the technique of the well-known Pfeiffer phenomenon the reader is referred to any standard work upon bacteriology. It is a very valuable corroborative procedure, but unnecessary for diagnostic purposes if the serum agglutination test can be applied. Its proper demonstration necessitates the use of a well-equipped laboratory.

Technique for the testing of the hæmotoxic or hæmolytic properties of vibrios and for making experiments with vibrios in the binding of complement are omitted. Scores of interesting experiments have been performed in these lines by Kolle, Meinicke, Schumacher, Mühlens and von Raven, Schütze, Weil, Markl, de Besche & Kon, Schottmüller, Kraus & Pribram, Ruffer, Göttschlich, and many others, but nothing to alter the demonstration of the absolute specificity of the agglutination reaction as first demonstrated beyond question by the classical work of Kolle. So that in spite of the interesting light thrown upon the biologic properties of vibrios, the discussion of these almost endless experiments is beyond the scope of a practical precis of this kind, and the interested reader may consult the original articles. Kolle and his coworkers proved the absolute specificity of agglutinating sera. He proved that serum prepared from a cholera vibrio agglutinated all cholera vibrios and had no more action on noncholera vibrios than normal serum in the same dilution. Also that an agglutinating serum prepared from a noncholera vibrio agglutinated that vibrio only, and had no effect whatever upon a true cholera vibrio. The writer was able in Manila to corroborate this

with a large number of freshly isolated cultures of both cholera and noncholera vibrios.

The use of the patients' serum tested against a known cholera organism for specific agglutinin or bacteriolysin is uncertain and unreliable as a means of diagnosis, consequently is not discussed here. The reader is referred to an interesting article by Svenson on this subject. (Zeitschrift für Hygiene, vol. 64, 1909.)

Discussion of the famous El Tor vibrio is avoided also. The literature on this one phase is enormous and the end is not yet. Suffice it to say that the consensus of opinion places the El Tor vibrios as true cholera vibrios, somewhat atypical in possessing toxic and haemolytic properties rarely found in cholera vibrios. However, Kolle, Meinicke, and others have shown that these properties are found occasionally in other cholera vibrios, and in view of the fact that these El Tor vibrios give the agglutination reaction and Pfeiffer's phenomenon with cholera sera they must be considered cholera vibrios. The persons carrying them without exhibiting any symptoms of cholera must be regarded as carriers or, as Pfeiffer has suggested, the vibrios for some reason may have lost their pathogenicity for man.

TREATMENT.

The treatment of Asiatic cholera may be considered under two heads, viz, treatment of collapse and treatment of uræmia.

Treatment of collapse.—The best treatment for collapse is the intravenous injection of salt solution. When feasible no other treatment for this condition is justifiable. The apparatus and technique are simple. Rogers recommended the use of hypertonic salt solution, on the ground that the use of this solution replaced not only fluid, but lost salts of the blood. The writer working with Dr. A. W. Sellards in Manila tested various salt solutions, including hypotonic and hypertonic solutions. The results showed equally beneficial effects from all in so far as judgment could be rendered from a series of about 100 cases.

The crying need of the patient is for fluid. This is needed primarily in the blood path. To inject into any other part of the body is a waste of very valuable time. Peritoneal or subcutaneous injections should only be employed when the number of patients, lack of time, or some other good reasons prevent intravenous injection.

Salt solution should be prepared and sterilized in 1 and 2 liter bottles. When needed it should be heated in a water bath to 43 to 45° C. A doubly perforated cork with one long glass tube to admit air and a short glass tube to which a sufficient length of rubber tubing is attached should be sterilized and kept in weak carbolic solution until needed. The following procedure is followed at San Lazaro Hospital in Manila:

The skin is cleansed over the internal saphenous vein above the internal malleolus, or one of the veins at the bend of the elbow. A small incision is made over the vein. The vein is dissected from the tissues and a grooved director passed under it. Two ligatures are placed one-half inch apart and the distal one tied. A small incision is made in the vein between the ligatures. A medium-sized canula is attached to the rubber tube of the transfusion apparatus and inserted into the vein after having allowed the fluid to flow through the canula

a few seconds. The bottle containing the salt solution described above should be reversed and hung about 4 feet above the bed, and the flow should not be too rapid, taking twenty to thirty minutes to inject 1,500 to 2,000 c. c. of fluid. The amount injected depends upon the condition of the patient. Usually 1,500 c. c. will be necessary and sometimes more to restore the fallen blood pressure and bring back the body heat. If collapse again supervenes within a few hours, the injection should be repeated, using one or the other ankle or forearm veins. Rogers very often leaves the canula tied in the vein for the use of a second injection. In Manila usually a different vein was used each time until both ankles and both elbows were bandaged. When a fifth injection is necessary the operation is similar to the first except that the incision is made one-half to 1 inch higher up, as described by Nichols and Andrews. After the operation the proximal ligature is tied and an antiseptic pad and bandage are applied.

The effect of intravenous injections in cholera is startling. It seems like resurrection, the body heat returns, the pulse becomes perceptible, then full and strong. If symptoms of collapse again appear, the operation must be repeated. Hot saline enemata have a good effect in washing out the lower bowel. The most important indication in the stage of collapse next to supplying the lost fluid is to conserve and maintain the body heat by hot bricks, hot-water bottles, blankets, etc. No nourishment should be given for the first thirty-six hours; nothing but cracked ice or small quantities of water. Rice-water broths or coffee may be given in small quantities after the second day. As convalescence begins, soft diet may be gradually introduced.

Treatment of the uræmia.—Treatment of the uræmia or the so-called cholera typhoid is the classical treatment of uræmia as described in any text-book. It has been suggested that this fatal complication was due to an acidosis, and on this theory the writer, with Dr. A. W. Sellards, of Manila, in December, 1909, substituted for the salt solution used intravenously a 2 per cent solution of sodium bicarbonate. The beneficial effects of fluid during cholera were apparently identical with those noted after the ordinary salt solutions, and in addition the incidence of uræmia following as a complication was reduced. The number of cases was not large enough to draw positive conclusions, and further experimentation is necessary.

PREVENTION OF CHOLERA.[a]

Before considering prophylactic measures it is necessary to consider how cholera is spread.

The infective agent in cholera is found only in the stools and vomit of persons who have in some way taken cholera organisms into their alimentary tract. The organisms may have been ingested directly into the stomach with food and drink, or at least the germs must have gained entrance to the mouth in some way.

Cholera is spread from place to place by individuals, carrying the cholera vibrios in their intestine and more or less sick with cholera.

[a] Prophylaxis of cholera by means of bacterial vaccines was first practiced by Ferran, developed by Haffkine, and improved by Kolle, Strong, and others. It seems to reduce the incidence of cases in a community. Its protection is not absolute and its sphere of usefulness is limited by popular dislike of inoculation procedures.

Where the distance between infected points is considerable the disease is probably carried by man, and by man alone.

Cholera is an absolutely preventable disease, and theoretically a case of cholera properly cared for should not result in further spread of the infection. The spread of cholera is primarily due to one of four factors:

1. Bacillus carriers.
2. Unrecognized light or atypical cases of cholera.
3. Failure to find or report cases early.
4. Carelessness in carrying out precautions, or failure to take such precautions.

The bacillus carrier.—The bacillus carrier is an individual carrying cholera vibrios in his intestine and yet who exhibits no signs of the disease.

The writer has never known a bacillus carrier to harbor cholera vibrios for longer than twenty days and the great majority lose their vibrios in less than ten days. However, many observers have found them present for longer periods, although all agree that the long-time carrier is the exception and not the rule. The following are the longest cited by Pfeiffer.[a]

Persistence of cholera vibrios in stools of convalescents, or bacillus carriers.

Name of observer.	Longest duration.	Name of observer.	Longest duration.
	Days.		*Days.*
Guttmann	10	Kolle	48
Lazarus and Pulicke	12	Donitz	49
Michailow	12	Abel and Clausen	15
Simonds	18	Pfeiffer	13
Rumpel	24	Bürgers[a]	69
Rommelaere	47		

[a] Hygienische Rundschau, February, 1910, Vol. XX, No. 4.

During times of epidemic bacillus carriers are numerous, and the writer found 6 to 7 per cent of carriers among healthy individuals living in the infected neighborhoods in Manila. When cases are few, the so-called sporadic cases, hundreds and even thousands of stools may be examined before the first carrier is found. The fact that the bacillus carrier may harbor the cholera vibrios as long as sixty-nine days illustrates how quarantines may be passed and an apparently inexplicable outbreak be explained. The danger from the bacillus carrier depends upon his habits and the sanitary conditions of the community in which he finds himself. If he deposits his stools in a modern flush closet in a city in which disposal of human excrement is properly effected and if he washes his hands frequently enough and at the proper time, he is harmless. His urine contains no vibrios. He may find himself, however, in a community with no proper system of disposal of excreta, or in spite of the existence of such system he may deposit his stool where flies or other insects have access thereto, or deposit it in a place from which a well or other source of water supply becomes infected. He may fail to wash his hands after defecation and with his dirty fingers infect the food or drink of others.

[a] Klinische Jahrbuch, 1908, vol. 19, p. 483.

In these ways the bacillus carrier is the greatest menace, and because of presenting no symptoms necessitates for our protection the safe disposal of the fæces of the entire population.

Mild or atypical cases.—Unrecognized, light, or atypical cases of cholera, or failure to carry out the necessary precautions, or carelessness in carrying out these precautions in recognized cases, are responsible for the spread of cholera, by permitting the infective material contained in the stools or vomit to get beyond control. Many writers speak of "latency" in cholera, "long incubation periods," etc., these terms indicating that an individual, for instance, a bacillus carrier, already carrying the vibrios in his intestine, may by reason of some factor which damages his intestinal mucosa or lowers his power of resistance, suddenly become ill after carrying the organisms for days beyond the ordinary period of incubation (one to five days). It is a very plausible theory, but lacks positive proof. I have seen cases which seemed to accord with this description, but was never able to exclude the possibility of infection from some unknown source (undiscovered bacillus carrier) within the ordinary incubation period.

A cholera stool improperly cared for may be deposited where flies and other insects may carry the vibrios to exposed food or drink. In communities without a safe water supply the stool may be deposited in or near a source of water supply. Milk may become contaminated either by flies or by washing the containers in infected water. Kitasato asserts that the vibrios will only live until the milk sours. There is some question about this, but in any event this duration of life would be quite long enough to permit milk to spread the disease.

Vegetables and fruits growing close to the ground are sometimes fertilized by human excrement. They may also be irrigated by infected water, and if eaten raw may thus be a means of spreading cholera.

In reviewing the manner in which cholera is spread, the prophylactic measures necessary are at once apparent. These will be discussed under two heads, viz: I, General preventive measures; II, Suppressive measures.

The first heading (General preventive measures) is intended to include those precautions which should be taken before the actual appearance of cholera in the community. Some of these, however, especially proper disposal of human excreta and the provision of a safe water supply, should be insisted upon by the health officer at any time on account of the constant danger of typhoid and other diseases, but especially when menaced by cholera.

GENERAL PREVENTIVE MEASURES.

1. Establishment of system of securing and recording information.
2. Organization of available personnel for sanitary work.
3. Enactment of necessary ordinances.
4. House to house inspection.
5. Safe disposal of feces of entire population.
6. Provision of a safe water supply.
7. Supervisory control of food and drink.
8. Campaign of education.

Securing, recording, and forwarding information.—Securing reliable information of the march of cholera is very necessary. The health

officer may obtain this information from the Surgeon-General, Public Health and Marine-Hospital Service, through the Public Health Reports, published weekly. Information of the entrance and spread of cholera within his State should be obtained from the state health officer and recorded carefully by the local health officer.

Information so received should be recorded upon maps of the State and municipality infected by means of flag-pins or pins with varicolored heads.

The local health officer should report daily to his state health officer the absence of cholera or if the disease be present, he should report the number of cases, and all pertinent information. Any suspicious diarrhea, especially in newly arrived persons, immigrants, or among those associating with such persons, should be treated with the same precautions as cholera and promptly reported to the state health officer and to the Surgeon-General of the Public Health and Marine-Hospital Service, Washington.

Organization of the sanitary personnel.—The sanitary personnel will necessarily depend upon the size of the municipality and the amount of money available. The health officer should at least have his plan of organization ready before the actual appearance of cholera. He should divide his municipality into districts. There should be a sanitary inspector for each district. The district should be of such size that the sanitary inspector could, if necessary, visit each house twice in a working day. He will need a fly-proof room for use as a morgue and should make provision for the possibility of having to isolate and care for cases of cholera or suspects. For disinfection he should have a unit of one disinfecting crew of two men with a wagon or cart. The number of crews will depend upon the size of the town and the number of cases of cholera. The local police may be used for inspection purposes and for the enforcement of health ordinances.

Enactment of ordinances.—Municipal ordinances should provide for the proper disposal of feces, the conservation of water supplies, prompt reporting of suspicious cases, collection and disposal of garbage, proper care of food and drink, and other sanitary necessities. If such ordinances are not in effect, it is the plain duty of the health officer to insist on their passage and to make sure that the penalties are adequate.

House to house inspections.—House to house inspection has a twofold object: (1) The finding of cases of suspicious illness; (2) to enforce sanitary maintenance of premises. This duty requires the maximum of courtesy and the minimum of words on the part of the inspector. A man without tact, courtesy, and patience must not be employed as a sanitary inspector. He should ascertain the number of persons in the house and leave a cholera circular upon his first visit. He should call attention to the necessity of protecting food and drink from flies or other sources of contamination. He should note the existence of garbage, refuse, filth, or any condition which favors the breeding or nourishment of flies. Cases of suspicious illness should be at once reported to the health officer, and at the end of the day a complete record of the number of premises inspected, insanitary conditions noted, etc. House to house inspection should be most carefully made in districts in which overcrowding or other insanitary conditions prevail and where arriving immigrants are apt to be found.

Disposal of feces and provision of a safe water supply.—Methods of disposal of feces and provision of a safe water supply will depend upon the size of the municipality and the funds available. Discussion of scientific and acceptable methods of disposal of feces and upon providing safe water supplies is beyond the scope of this precis. The health officer, from works upon hygiene and sanitary engineering, can select the systems best adapted for his municipality. If he is compelled to permit the more primitive methods from reasons of economy, he can at least insist upon protecting his shallow wells from pollution and upon making his primitive closets fly-proof.

Supervisory control of food and drink.—The health officer personally or through his sanitary inspectors should exercise the closest supervision over markets, stores, restaurants, hotels, and other places where food and drink are manufactured or exposed for sale. Unnecessary, careless, or uncleanly handling of foodstuffs should be prevented and all prepared foodstuffs protected from flies and other insects.

Campaign of education.—The success of cholera prophylaxis depends largely upon popular education. The health officer, through the schools, through popular meetings, and by means of circulars, should disseminate knowledge of cholera in simple language among the people, showing them how they may protect themselves from infection. A popular circular may be distributed based upon the following:

Cholera circular.

Cholera CAN BE INTRODUCED INTO THE SYSTEM ONLY THROUGH THE MOUTH. It is caused by organisms too minute to be seen except with a microscope. These organisms are readily killed by heat, and the disease may therefore be successfully combated by the proper use of fire and hot water, which are at the disposal of everyone.

To avoid cholera and prevent its spread observe the following precautions:

1. Boil all drinking water and place it while hot in covered vessels. Do not dip up the water when needed, but POUR it into drinking cups; otherwise cholera germs may get into the water from the hands.
2. Do not touch drinking water or food with the hands unless they have just been washed in water that has been boiled.
3. Eat only cooked food. Avoid all raw fruits and vegetables. Fruits may be made comparatively safe by dipping them a few seconds into boiling water.
4. Flies may carry cholera germs on their feet from human excreta to food; therefore, to protect it from flies, cover all food immediately after it is cooked.
5. Boil all water used for diluting milk.
6. Cook all meats and fish thoroughly so as to heat the same throughout.
7. Keep kitchen and table dishes thoroughly clean and scald them before using.
8. Keep the place in which you live, the ground under the house, and everything pertaining to it, clean.
9. Outhouses, closets, and vaults can be made safe by putting in lime or carbolic acid. When this can not be done dejecta may be buried or thoroughly covered with earth.
10. Isolate all the sick.
11. Filth or vomit and the dejecta of the sick should be promptly cleaned up with boiling water and buried.
12. Clothes and bedding used by sick persons must be boiled. Do not wash any clothes near wells or springs nor permit surface water to run into any well or spring.

SUPPRESSIVE MEASURES.

Under suppressive measures which are imperative after cholera has appeared in the municipality, must be considered the following: 1, Early discovery of cases; 2, isolation and care of patient; 3, disinfection; 4, observation of contacts and precautions to be taken with them.

Early discovery of cases.—Early discovery of cases is the measure of greatest importance in the suppression of a cholera outbreak.

Ordinances should exist requiring the prompt reporting of suspicious diarrheas, and placing the obligation for reporting such cases upon householders, hotel or boarding-house keepers, nearest relatives, and attending physicians. Much depends upon the attitude of the local profession and the alacrity or tardiness with which they respond to this duty.

Careful watch over death certificates and autopsies upon those dead under suspicious circumstances is essential. Sometimes, instead of complete autopsy an abdominal incision and removal of a portion of small intestine suffices, and consent therefor is obtained with less trouble. Special attention must be paid to the foreign quarters and newly arrived immigrants, if such exist.

Isolation of the patient.—A patient with cholera or suspected of having cholera should be isolated immediately. The room or ward should be rendered fly-proof by screening. In the room with the patient there should be a tub or other large vessel containing 5 per cent solution of carbolic acid crystals for the immediate reception of soiled linen.

The stools and vomit of the patient should be disinfected at once by adding an equal volume of 5 per cent carbolic acid solution, 5 per cent formaldehyde solution, or milk of lime. The mixture should be covered and allowed to stand for two hours before ultimate disposal. There should also be a washstand and basin just inside the door of the room and every person before leaving the room should be required to thoroughly wash and disinfect the hands with a 1 per cent solution of lysol or other good disinfectant.

Gowns should be put on upon entering the sick room and should be taken off just before disinfecting the hands and leaving the room. These gowns when soiled should be placed with other soiled linen in the tub of carbolic acid solution.

Disinfection.—There should be a thorough surface disinfection of *every room in the house* in which a case of cholera or suspected cholera is found.

The infection of cholera is not air-borne and is not likely to be found higher than a man can reach, so that this disinfection is effectively secured by mechanical cleansing of the walls and floor with disinfecting solution, (2½ per cent carbolic acid, 1 to 1,000 bichloride solution). This disinfection should not only be performed after the death or removal of a patient, but of course should be more or less continuously carried out in the sick room or hospital ward by mopping of the floor and washing or spraying the walls with the disinfectant solutions above described.

The cholera organism is easily killed by drying and by heat, and infected objects may either be immersed in 5 per cent formalin or 5 per cent carbolic acid solution, or disinfected by dry heat or boiling water.

It will be necessary sometimes to disinfect rooms containing objects and fabrics which would be ruined by immersion or boiling. These rooms should be disinfected by formaldehyde gas. Bichloride solution corrodes metals and such objects should be boiled or immersed in one of the other solutions. All remnants of food about a cholera house should be destroyed by burning. Drinking water or other beverages should be disinfected and disposed of. Cutlery, kitchen

utensils, crockery, etc., are best disinfected by boiling. Outside of the house where to disinfect is determined by the possibility of the object or place being infected with fecal material and the existence of moisture.

Observation of contacts and precautions to be taken with them.—After isolation of the patient and disinfection of the premises, the contacts or persons who have been in contact with the sick one must be cared for.

The hands of the contacts and such clothing as may have been exposed to infection must be disinfected, and the contact visited twice daily for a period of five days. During these five days there should be at least two examinations of the stools of each contact, one as soon as possible after discovery of the initial case and the other before discharge from observation. Should either of these examinations prove positive for cholera vibrios the contact must be isolated at once and the same precautions taken as in any other case of cholera. Until two vibrio-negative reports are received stools of contacts and their hands are to be disinfected precisely as in actual cholera cases.

Convalescents should have three vibrio-negative reports of stools examined on successive days and should never be discharged upon one single vibrio-negative report.

APPENDIX.

I. *Nutrient bouillon.*

One-half kilogram beef, free from fat, is cut in very small pieces and allowed to stand with 1 liter of water twenty-four hours in the ice chest or for one hour in the incubator at 37° C. Press through cheese cloth. Add water up to 1 liter, add 10 grams Witte's peptone and 5 grams salt. Cook for one-half hour. Make alkaline with solution of caustic soda. Heat again three-fourths hour and filter.

II. *Cholera agar.*

Take 1 liter of nutrient bouillon (No. I) and add 30 grams agar, dissolve by heat and alkalinize with caustic soda solution. To reach a desirable grade of alkalinity in cholera media, add 3 c.c. of a 10 per cent caustic soda solution to each 100 c.c. of media which is neutral to litmus. The agar should be sterilized in tubes containing 15 c.c. each.

III. *Cholera peptone solution.*

Peptone (Chapoteau or Witte)	10.0
Salt	10.0
Potassium nitrate	.1
Sodium carbonate	.2
Distilled water	1,000.0

Dissolve by heat, filter, and sterilize in tubes containing 15 c.c. and flasks containing 50 c.c. for use.

IV. *Alkaline blood agar medium of Dieudonne.*

Defibrinated ox blood	30
Normal solution of caustic potash	30
Cholera agar (No. II)	140

Add the caustic potash solution to the ox blood, and add the melted agar. Sterilize for one hour at 100° C., and use about 15 to 20 c.c. for each plate.

V. *To prepare an agglutinating cholera serum.*

Use eighteen-hour cultures of a known cholera vibrio upon agar and inject in the ear vein of a rabbit a suspension of the organism in salt solution which has been heated for one hour at 60° C.: First day, 1 loop; seventh day, 3 loops; fourteenth day, 5 loops; twenty-first day, 1 slant (about 8 loops).

The fourth injection may be given intraperitoneally and the rabbit is ready to bleed on the twenty-eighth day. This procedure usually gives a serum with a titer of 1 to 4,000.